REMARKS

This correspondence responds to the Office Action which was mailed on April 24, 2008. No new amendments to the specification or claims have been made herein. In light of the arguments and remarks set forth below it is respectfully submitted that Claims 15, 23 and 24 are in condition for allowance. Applicant requests a favorable reconsideration of this application in light of the remarks set forth below which constitute a full and complete response to the outstanding Office Action.

Claims 15, 23 and 24 were rejected under 35 U.S.C. §103(a) as being unpatentable over the publications of Letertre et al. in light of O'Connell et al., further in view of Borst et al., and further in view of Padmapriya et al. Letertre and O'Connell were relied on to teach the method steps of real-time fluorescence PCR, while Borst was relied on to teach primers "100% identical to SEQ ID NO: 3, 4 and SEQ ID NO: 5, 6 (see page 5423 and sequence alignment #STAENAB for SEQ ID NO: 3 and NO: 5.) It was also asserted Borst teaches "primers and probes in the size of 20 to 21 bases in length from an entire sea gene having a length of 1443 bases (see Borst et al. pages 5422-5423 and sequence alignments for SEQ ID # 3, 4 and SEQ ID #5, 6)." Finally, it was asserted that Padmapriya teaches "primers of size of 20 or more nucleotides 100% identical to SEQ ID NO:1 and 2 which target *Staphylococcus* enterotoxin A gene and used in detecting Staphylococcus aureus causing food poisoning (see abstract, claims and sequence alignment # AED45640)." It is respectfully submitted that this argument is untenable and should be withdrawn because Borst and Padmapriya do not actually teach primers identical to those disclosed and claimed by applicant, and therefore, the combination of Letertre, O'Connell, Borst and Padmapriya does not teach the primers and probes claimed by applicant in the claimed method.

More specifically, the primer sequences taught in Borst are simply not designed for real-time PCR and differ from applicant's primers in several respects. First, the primer sequences taught in Borst amplify a sequence of 272 base pairs in length. On the other hand, target sequences that are optimal for real-time fluorogenic PCR are in the range of 50-150 base pairs in length. Accordingly, applicant's primers amplify sequences of 101 and 99 base pairs in length. In addition, Borst simply does not teach primers with sequences identical to those claimed by applicant. Borst teaches an upstream primer of 5' AGCATACTGCAAGTGAAGTTG 3'and a downstream primer of 5' TTGTTGTCAACGTTAGGG 3'. These sequences are not matches with the primer sequences claimed by applicant in Claim 15. It is believed that any match found to applicant's sequences probably resulted from the fact that the entire sequence of Accession L22565 was referred to in the Borst publication. L22565 comprises the entire sequence of accession for the upstream region of the *sea* gene. Therefore, it may include as part of the entire sequence those potions identified by applicant as SEQ ID NO: 3 and SEQ ID NO: 5, but that does not constitute a teaching of those specific primer sequences which are only a small part (20 or 21 bases) of the L22565 Accession. Furthermore, the majority of SEQ IDS which are claimed and disclosed by applicant are not included in the L22565 Accession at all since it does not include the entire *sea* gene. Applicant has claimed specific primers and probes of about 20 or 21 bases in length from an entire *sea* gene having a length of 1443 bases.

It is also important to note that while Claim 15 uses the open transitional term "comprising" which is open-ended with respect to the overall method steps, it also includes as limitations "a primer selected from the group *consisting of* a forward primer having a

specific sequence selected from the group *consisting of* SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence selected from the group *consisting of* SEQ ID NO: 5, SEQ ID NO: 6 and combinations thereof" and "wherein the nucleic acid sequence of the nucleic acid probe is selected from the group *consisting of* SEQ ID NO: 1, SEQ ID NO: 2." Thus, the specific primers and probes used in applicant's claimed method are specifically limited to the exact sequence listings recited by applicant.

Furthermore, the Borst sequences violate several of the primer set design guidelines that must be followed to obtain a set of primer and probe oligonucleotide sequences that will perform optimally in real-time fluorogenic PCR. Specifically, the two primers described in Borst are not suitable for use in identifying the *entA* gene by real-time fluorogenic PCR for several reasons, including: (a) the amplicon being of 272 base pairs while real-time fluorogenic PCR optimally requires 50-150 base pairs; (b) Borst does not teach the melting temperature of their primer pair, and primer pairs designed for optimal performance in real-time fluorogenic PCR have melting temperatures between 58 and 60 degrees C; and (c) the downstream primer sequence taught in Borst violates the guideline for primer selection that no more than two of the five bases at the 3' end of a primer be either G or C (Borst teaches a downstream primer with three G/C bases among the five bases at the 3' end of the primer).

Moreover, only one of the primers taught by Borst (that appearing in the text on p. 5423) binds a sequence inside the open reading frame encoding the SEA protein. The other primer binds a sequence upstream of the promoter for the *entA* gene. The use of this primer pair will fail to detect the gene if the gene has been excised from its native sequence and cloned behind a promoter that has been optimized for expression of the gene in another organism (as may be the case if the gene is used to create a genetically engineered biological

weapon). Therefore, in addition to not being suitable for real-time PCR, the primers described on pages 5422 and 5423 do not constitute a functioning assay directed specifically and solely at sequences that encode staphylococcal enterotoxin A.

Furthermore, the primer sequences taught in Padmapriya are not the same as applicant's claimed SEQ ID No: 1 and SEQ ID NO: 2. They simply are not 100% identical. Close inspection will reveal that Padmapriya's forward primer overlaps an internal portion of applicant's *probe* sequence SEQ ID: NO 1, not a primer sequence. Likewise, a close inspection of Padmapriya's reverse primer sequence reveals that it is not identical to or overlapping any of applicant's primer or probe sequences SEQ ID NO: 1 through 6.

In addition, the size of the amplicon resulting from the use of Padmapriya's primers is 301 bases in length, which is more than double the optimal size for real-time PCR, and therefore, can not be combined with the teachings of Borst et al. or Letertre et al. to make optimal real-time fluorescent PCR assays. Finally, the combination of prior art cited does not specify probe sequences at all.

Applicant readily acknowledges that the general practice of real-time fluorogenic PCR is well established among molecular biologists. However, the assay that is the subject of this patent application is a specific, non-obvious application of the technique designed to detect the presence of nucleic acids encoding the *entA* gene in a sample. The unique, non-obvious feature of the assay is the sequences selected to form the primer and probe oligonucleotides one would use in the performance of the assay.

Applicant reiterates that the selection of specific sequences for PCR primers and probes is not obvious. A researcher should not expect optimal results, or indeed, any results at all, by merely selecting random sequences 20 bases in length to serve as forward and

reverse primers. All workers who design PCR assays must obtain several primer pairs and test them empirically to determine which pairs work well (if any at all) under the desired reaction conditions. While Letertre et al. do describe PCR assays that detect the *entA* gene, the assays described are up to 25-fold less sensitive than applicant's assays and are clearly not identical.

In a previous Office Action the examiner noted that one can not show non-obviousness by attacking references individually where the rejection is based on the combination of references. However, where individual references are relied on as teaching particular elements/limitations in applicant's claims, applicant is permitted to argue that those individual references do not actually teach what the examiner is asserting they teach, and therefore, that a *prima facie* case of obviousness has not been established. Such is the case here, where applicant is arguing that Borst and Padmapriya do not, in fact, teach primers and probes which are 100% identical to those of SEQ ID NOs: 1-6 as claimed in Claim 15.

In summary, Claims 15, 23 and 24 remain in the case and based on the foregoing remarks should not be considered obvious in view of the prior art cited. Accordingly, it is respectfully submitted that these claims are patentable and in condition for allowance. Early reconsideration and withdrawal of the rejections is earnestly solicited, as is allowance of the claimed subject matter.

Respectfully submitted,

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DATE

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